

SUPPLEMENTARY INFORMATION

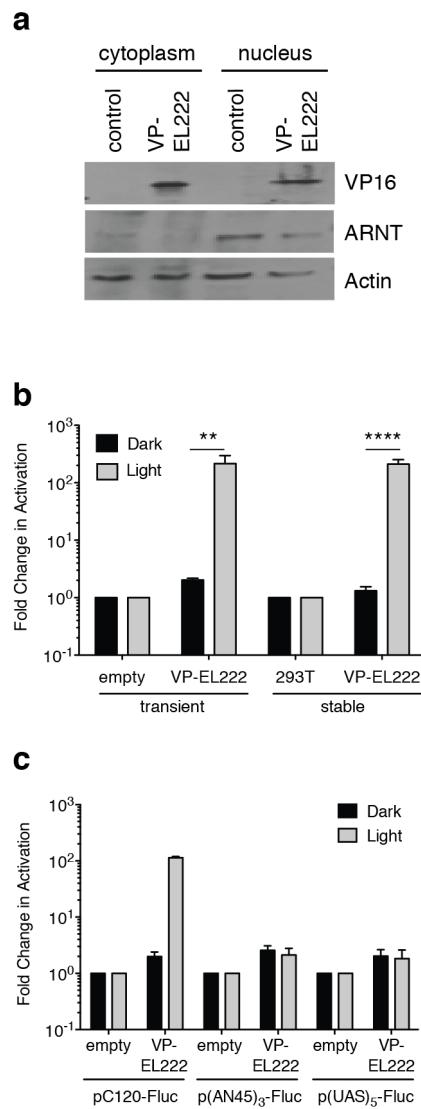
An optogenetic gene expression system with rapid activation and deactivation kinetics

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This supplementary information contains the Supplementary Figures 1-5, Supplementary Tables,
legends for Supplementary Videos, Supplementary Notes, and Supplementary References.

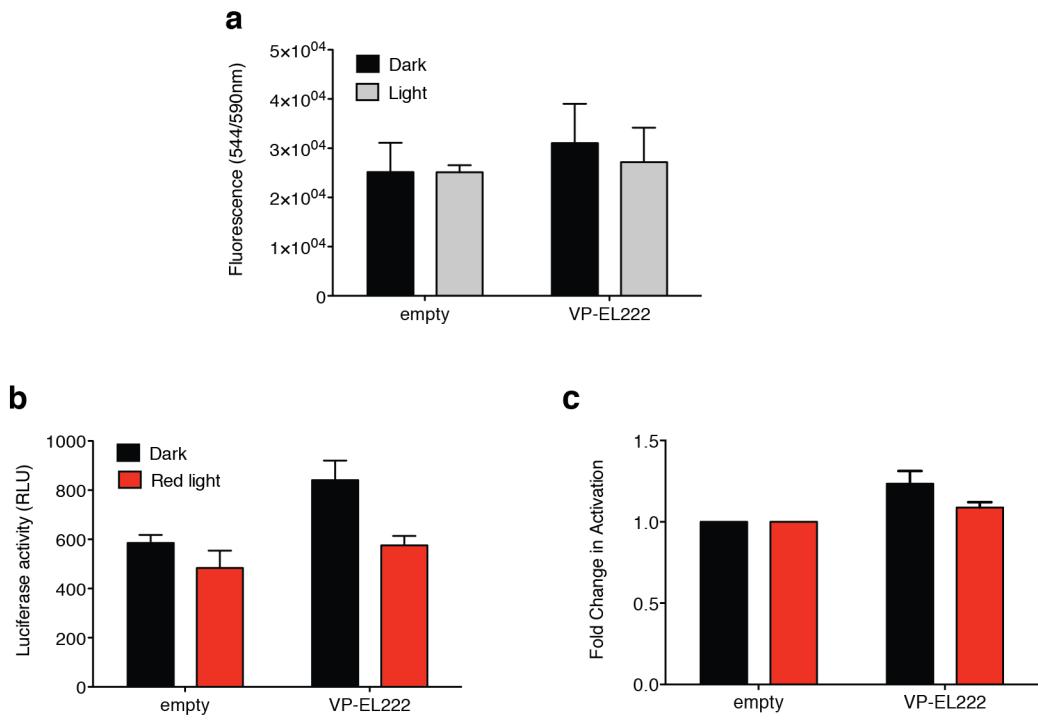
SUPPLEMENTARY RESULTS

SUPPLEMENTARY FIGURES

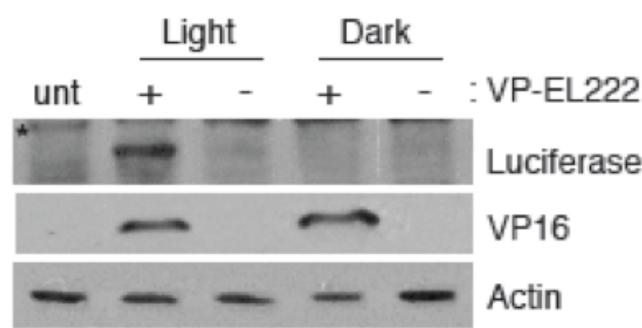


Supplementary Figure 1. VP-EL222 is expressed in 293T cells and can function to activate luciferase expression in response to light. (a) Western blot analysis of VP-EL222 expression in cytoplasmic and nuclear fractions made from 293T cells transiently transfected with pVP-EL222. An ARNT antibody was used as a marker for nuclear localization. (b) The fold change (FC) in transcription in control versus VP-EL222 cells under dark and light conditions was

calculated by normalizing the luciferase levels (**Fig. 1**) to *Renilla* luciferase levels [$FC_{\text{dark or light}} = (\text{Firefly}/\text{Renilla})_{\text{VP-EL222}} / (\text{Firefly}/\text{Renilla})_{\text{empty or 293T}}$]. ($n = 3$ independent experiments, each performed in triplicate per condition).

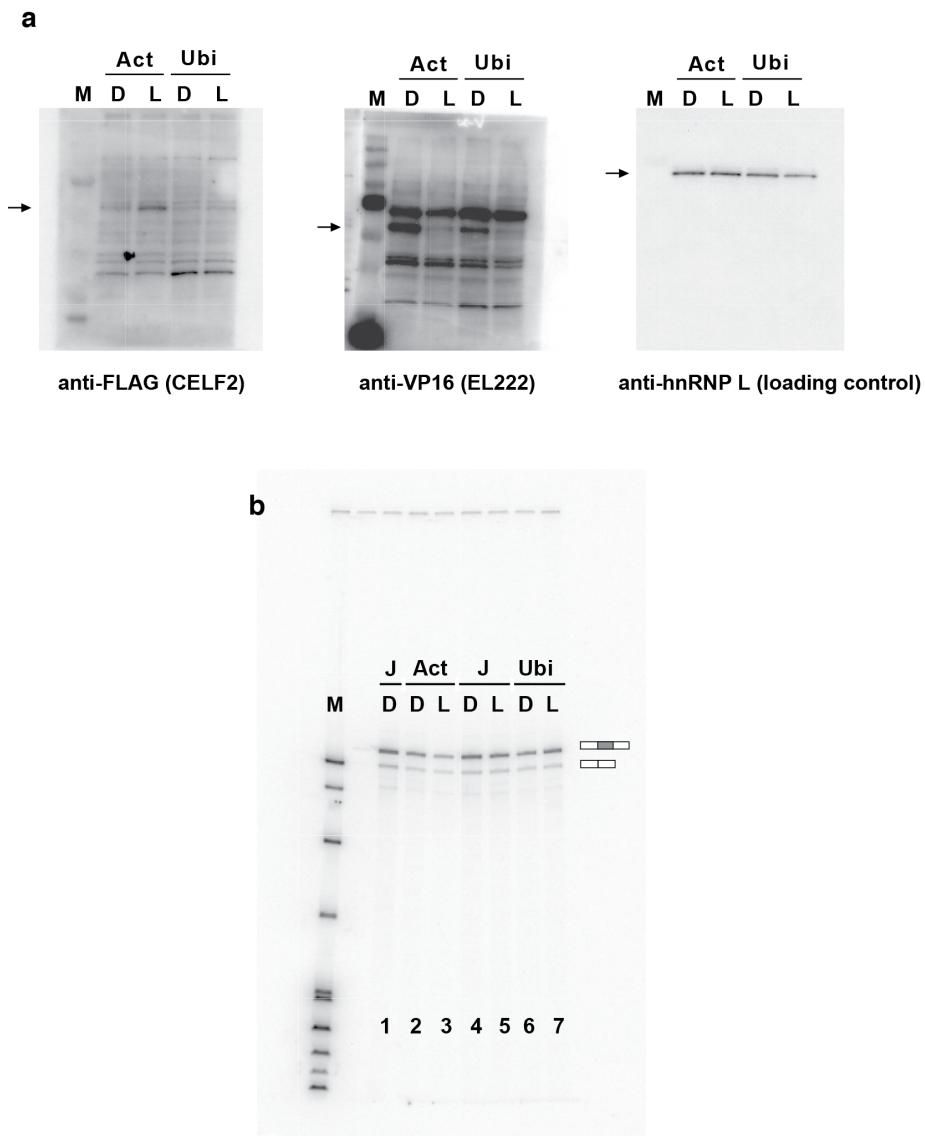


Supplementary Figure 2. VP-EL222 is not activated by red light and that illumination with blue light has no negative effect on cell viability. (a) Results of cell viability assays using CellTiter-Blue reagent. 293T cells were transiently transfected with either empty vector or pVP-EL222 and pC120-Fluc and kept in the dark or illuminated with pulsing blue light (20 s on, 60 s off) for 24 hr. Subsequently, the cells were incubated with CellTiter-Blue for 2 hr and the resultant fluorescence ($544_{\text{Ex}}/590_{\text{Em}}$) was recorded ($n = 2$ independent experiments, each performed with six replicates per condition). **(b, c)** 293T cells were transiently transfected with either empty vector or pVP-EL222 and pC120-Fluc, at 24 hr post-transfection cells were kept in the dark or illuminated with continuous red light for 24 hr. Afterwards, luciferase levels were measured **(c)** and then used to calculate the fold change in gene expression **(d)** in cells expressing VP-EL222 versus empty vector control ($n = 1$ independent experiment performed with three replicates per condition). All data are represented as mean \pm s.d.



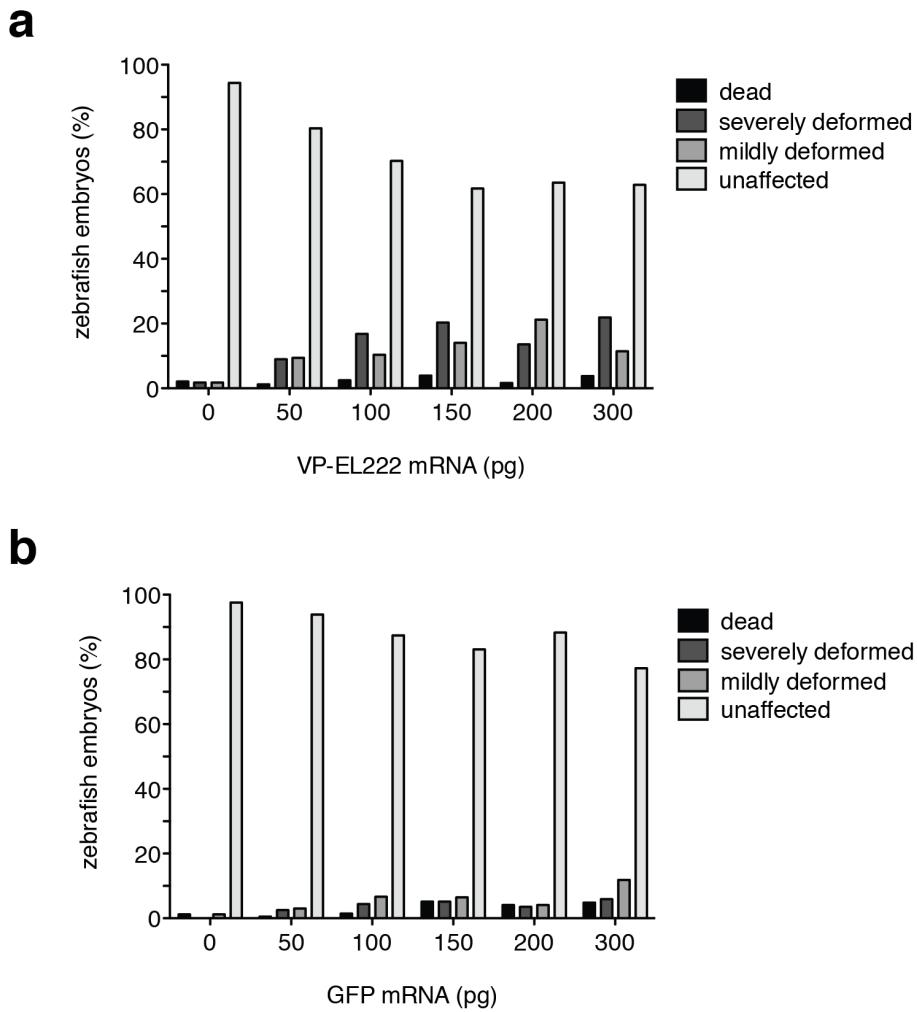
Supplementary Figure 3. Light-triggered expression of luciferase protein by VP-EL222.

Western blot analysis of luciferase expression in VP-EL222 cells (+) or wild-type 293T cells (-) after illuminating with blue light or kept in the dark. Untransfected control (unt).



Supplementary Figure 4. Full Western blots and denaturing polyacrylamide gel of RT-PCR samples. (a) Blots were probed with antibodies against FLAG, VP16, and hnRNP L. EL222-VP16 was driven from an Actin promoter (Act; used in **Fig. 4a**) or Ubiquitin promoter (Ubi, not used in subsequent studies). Arrow indicates band of interest. (b) RT-PCR to quantify splicing of endogenous CELF2. Lane 1, wild-type JSL1 cells. Lanes 2-7, JSL1 cells containing VP16-EL222 driven from Actin promoter (Act). Lanes 4-5, wild-type JSL1 cells. Lanes 6-7, JSL1 cells containing VP16-EL222 driven from Ubiquitin promoter (Ubi). Lanes 2-5 are shown

in **Fig. 4b** (lanes 2-3, right side of panel; lanes 4-5, left side of panel). Dark conditions (D); Light conditions (L); Molecular weight marker (M).



Supplementary Figure 5. VP-EL222 is only moderately toxic in zebrafish. Dose-response curve showing **(a)** the effect of VP-EL222 mRNA expression on zebrafish development as compared to **(b)** expression of a control GFP mRNA (right). Embryos were injected at the one to two-cell stage and illuminated with constant blue light until they were scored at 24 h.p.f. (at least $n = 100$ embryos per condition).

SUPPLEMENTARY TABLES

Supplementary Table 1. Occurrence of C120 sequence in human, mouse and zebrafish

genomes. The BLASTN 2.2.28+[3] program was used to search the indicated nucleotide databases for the EL222 binding site Clone-1 20 bp (C120) sequence. For comparison, the same databases were searched for the GAL4-specific Upstream Activation Sequence (UAS). To identify the top hits in each search, we chose a cutoff Expect (E) value < 10 and required that the sequences have no gaps or mismatches to original query sequence.

Search sequences:

C120 (20 bp) TAGGTAGCCTTAGTCCATG

UAS (20 bp) GGAGGACAGTACTCCGCTCg* (one extra base added to make 20 bp query)

Organism	Database searched	# of sequences in database	C120 # of hits (E value <1000 / E value <10)	UAS # of hits (E value <1000 / E value <10)
<i>Homo sapiens</i>	Ref Seq Genomic	22,540	200 / 14	426 / 7
<i>Mus musculus</i>	Ref Seq Genomic	20,034	200 / 22	486 / 0
<i>Danio rerio</i>	Genome (ref only)	4,560	217 / 6	131 / 0

Top hits with E value <10, no mismatches, no gaps

Organism	C120	UAS
<i>Homo sapiens</i>	8 hits match 17 bp	7 hits match 17 bp
<i>Mus musculus</i>	18 hits match 16 bp	0 hits match 15 bp
<i>Danio rerio</i>	6 hits match 15 bp	0 hits match 14 bp

SUPPLEMENTARY VIDEOS

Supplementary Video 1. Z-stack of 70% epiboly embryo showing mosaic expression of mCherry after illumination with blue light. Representative zebrafish embryo after injection with both VP-EL222 mRNA and pC120-mCherry DNA and illumination with constant blue light for 5 hr beginning at 2 h.p.f. Fluorescent and brightfield images were acquired every 2.58 μ m on a Digital Scanned Laser Light Sheet Microscope[1]. The two channels were merged and the z-stack converted into a video on ImageJ[2]. Mosaic expression is due to random incorporation of the pC120-mCherry DNA into cells as the embryo develops.

Supplementary Video 2. Z-stack of 70% epiboly embryo showing no expression of mCherry under dark conditions. Representative zebrafish embryo after injection with both VP-EL222 mRNA and pC120-mCherry DNA and kept in the dark for 7 hr. Fluorescent and brightfield images were acquired every 2.58 μ m on a Digital Scanned Laser Light Sheet Microscope[1]. The two channels were merged and the z-stack converted into a video on ImageJ[2].

Supplementary Video 3. Localization of fluorescent mCherry in the heart of a developing zebrafish embryo at 24 h.p.f. Representative zebrafish embryo's heart after injection of both pminiTol2-*myl7*-VP-EL222-C120-mCherry DNA and transposase mRNA and illumination with constant blue light for 14 hr beginning at 10 h.p.f. Fluorescent images of a single plane within the zebrafish heart were acquired by time-lapse epifluorescent microscopy (Nikon Ti-E). Frames were acquired every 144 ms for 10 s. Playback is 7 frames/s.

SUPPLEMENTARY NOTES

Supplementary Note 1. Algorithm for kinetic modeling of VP-EL222 activation

parameters. To examine the dependence of EL222-based transcriptional activation on properties of the engineered VP-EL222 protein, we developed a conceptual model for the effect of pulsed blue light activation on EL222-driven transcription. We assumed that each pulse of light triggers 3 phases of gene expression (Fig. 3a):

- 1). A sigmoidal buildup phase characterized by EL222 activation and dimerization, DNA binding and transcriptional activation through the point of RNA Pol II promoter clearance, described by the collective rate constant τ_{on} . We checked for the need for cooperativity in this process, as implemented by Hill coefficients (h) between 1-5; while it was essential to have some degree of cooperativity ($h > 1$), we found minimal variation in the quality of fitting our experimental data for values between 2-5. Of these, $h=4$ provided the most optimal fit and was chosen for these simulations.
- 2). Once $t > \tau_{\text{on}}$, transcriptional activity saturates and enters a steady state phase, where transcription occurs at a maximal rate for as long as the cell is illuminated.
- 3). Once illumination ceases, active VP-EL222 decays as a first order exponential at a rate of τ_{off} , a process we assume to be likely dominated by cleavage of the cysteine/flavin adduct within the EL222 LOV domain. Transcriptional activity falls subsequently as EL222 reverts to the monomeric dark state, free from DNA.

For the purposes of the simulation, luciferase activity was taken to reflect the sum of all transcriptional activity over time, where each EL222 binding event generates one or more

luciferase mRNAs provided that it remains on the promoter long enough for RNA polymerase commitment to generating a full-length transcript. In order for transcriptional activity to move away from initialized values and reach steady state, the model was allowed to run for 10 light-dark cycles, each 80 s long, with an illumination time between 0 and 20 s. The EL222 concentration was assumed to be at steady-state and unaffected during these ten cycles. Predicted luciferase activities for a given τ_{on} , τ_{off} pair were normalized and compared to experimental values. For illumination times of 0, 2, 5, 10 and 20 s, the difference between the predicted and actual value for luciferase activity was computed and squared (Fig. 3b). The sum of these five errors was assumed to reflect the overall quality of the prediction. To determine τ_{on} , τ_{off} pairs that gave the least squared error, a simple grid search was implemented in MATLAB version R2012a (code provided as Supplementary Note 2), where the error function was evaluated at all combinations of τ_{on} and τ_{off} for values of $\tau_{on} = 1\text{-}100$ s and $\tau_{off} = 1\text{-}100$ s (Fig. 3e shows expansion of values between $\tau_{on} = 1\text{-}10$ s and $\tau_{off} = 1\text{-}100$ s).

Supplementary Note 2. MATLAB code for kinetic model (provided as a separate file).

Supplementary Note 3. DNA and plasmid sequence information.

AN45[4] GGCCCCGAGGTCCAGCACCAACGCAGTCCCCTTGGTACGCCGAC
C120[5] TAGGTAGCCTTAGTCCATG

1. p(AN45)₃-Fluc construct

This construct was used for transient transfections. Three copies of the 45 bp version of the AN45 DNA sequence were cloned into the pGL4.23 vector (Promega) using XhoI (5'end) and HindIII (3'end).

Bold= restriction sites

Underlined= AN45 repeats

Yellow= minimal TATA-box promoter

Green= Firefly Luciferase open reading frame

Vector sequence:

GGCCTAACTGGCCGGTACCTGAGCTCGCTAGC**CTCGAG**GTCGACGGCCCCGAGGTCCAGCACCAACGCAGTCCCCTTGGTACGCCGAC**AAGCTTA**
GACACTAGAGGGTATATAATGGAAGCTCGACTTCCAGCTTGGCAATCCGGTACTGTTGGTAAAG
CCACCATGGAAGATGCCAAAAACATTAAAGAAGGGCCAGCGCCATTCTACCCACTCGAAGACGG
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 CTAGCAAATAGGCTGCCAGTGCAGGTGCCAGAACATTCTCT

2. pcDNA-C120-empty construct

Five copies of the 20 bp version of the Clone-1 DNA sequence were cloned into was cloned into the pcDNA3.1(+) vector (Invitrogen) using BlgII (5'end) and BamHI (3'end).

Bold= restriction sites

Underlined= C120 repeats

Yellow= minimal TATA-box promoter

Vector sequence:

GACGGATCGGG**A****G****A****T****C**TCGCTAGCCTCGAGTAGGTAGGCCTTACTGCATGCGTTAGGTAGC
CTTAGTCATGCGTTAGGTAGCCTTACTGCATGCGTTAGGTAGCCTTACTGCATGCG
TTATAGGTAGCCTTACTGCATGAAGCTT**A****G****A****C****A****T****A****G****G****G****T****A****T****A****T****G****G****A****A****G****C****T****G****A****C****T****C**
C**A****G****C****T****G****G****C****A****T****C****C****G****T****A****C****T****G****T****G****T****A****A****G****A****G****A****T****C**
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3. pC120-Fluc construct

For the purposes of clarity, the two reporter gene constructs (pGL4.23-C120-Fluc and pcDNA-C120-Fluc) used in this study are both referred to as pC120-Fluc in the text. Nevertheless, the specific experiments in which each construct was used are clearly listed in the Methods section. In general, pGL4.23-C120-Fluc was used for transient transfections, and pcDNA-C120-Fluc was used for transfection of VP-EL222 stable cell line. The sequences for both constructs are listed below.

a) pGL4.23-C120-Fluc

This construct was used for transient transfections. Five copies of the 20 bp version of the Clone-

1 DNA sequence were cloned into the pGL4.23 vector (Promega) using XhoI (5'end) and HindIII (3'end).

Bold= restriction sites

Underlined= C120 repeats

Yellow= minimal TATA-box promoter

Green= Firefly Luciferase open reading frame

Vector sequence:

GGCCTA**ACTGGCCGGTAC**TGAGCTCGCTAGC**CTCGAG**TAGGTAGCCTTAGTCCATGCGTTAT
AGGTAGCCTTAGTCCATGCGTTTAGGTAGCCTTAGTCCATGCGTTTAGGTAGCCTTAGT
CCATGCGTTTAGGTAGCCTTAGTCCATG**AAGCTT**AGACACTAGAGGGTATATAATGGAAGCT
CGACTTCCAGCTTGCAATCCGGTACTGTGTAAGGCCACCATGGAAGATGCCAAAAACATTA
AGAAGGGCCCAGGCCATTCTACCCACTCGAAGACGGGACGGCCGGCGGAGCAGCTGCACAAAGC
CATGAAGCGCTACGCCTGGTGCCCGGCACCATGCCTTACCGACGCACATATGAGGTGGAC
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GCATCCTGCTGCAAACACCCAAACATCTCGACGCCGGGTCGCCGGCTGCCGACGACGATGC
CGCGAGCTGCCCGCAGTCGTCGTGTGGAAACACGGTAAAACCATGACCGAGAAGGAGATC
GTGGACTATGGGCCAGCCAGGGTACAACCGCCAAAGAAGCTGCCGGTGTGGTGTTCGTGG
ACGGAGGTGCCTAAAGGACGTGACCGGAAGTTGGACGCCCCGAAGATCCCGGAGATCTCATTAA
GGCCAAGAAGGGCGGAAGATCGCCGTTAATAATTCTAGAGTCGGGGCGCCGGCGCTTCGA
GCAGACATGATAAGATACATTGATGAGTTGGACAAACCACAAACTAGAATGCAGTGAAAAAAAT
GCTTATTGTGAAAATTGGTATGCTATTGCTTTATTGTAACCATTATAAGCTGCAATAAACA
AGTTAACAAACACAATTGCATTGCTTTATGTTTCAGGTTCAGGGGAGGTGTGGGAGGTTTT
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AGAGCCTCAACCCAGTCAGCTCCTCCGGGTGGCGCGGGGGCATGACTATCGCGCCGCACTTA
TGACTGTCTCTTATCATGCAACTCGTAGGACAGGGTUCCGGCAGGCGTCTTCCGCTCTCG
TCACTGACTCGCTCGCCTCGGTCGTTCGGGCTCGGGCGAGCGGGTATCAGCTCACTCAAAGGGGT
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CTGTCCGCCTTCTCCCTCGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCA
GTTCGGTAGGTGCTCCAAGCTGGCTGTGACGAACCCCCGTTAGCCGACCG
CTGCGCCTTATCCGTAACATCGTCTTGAGTCCAACCCGTAAGACACGACTTATGCCACTG
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AGTTACCTCGAAAAAGAGTTGGTAGCTCTGATCCGGCAAACAAACCACCGCTGGTAGCGGT
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TCCTTCTACGGGTCTGACGCTAGTGGAACGAAAACACGTTAAGGGATTTGGTATGAG
ATTATCAAAAGGATCTCACCTAGATCCTTAAATTAAAAATGAAGTTTAAATCAATCTAA
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CTCCCCGTCGTAGATCACTACGATTGAGGGCTTACCATCAGGCCAGCGCAGCAATGA
TGCGCGAGAGCCGCGTCACCGGCCCGATTGTCAGCAATGAACCAGCCAGCAGGGAGGGC
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GCTAGAGTAAGAAGTCGCCAGTGAGTAGTTCCGAAGAGTTGTGGCCATTGCTACTGGCATCG
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TGCCATCCGTAAGATGCTTCCGTGACCGGGAGTACTCAACCAAGTGTGAGTAGTG
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ACTTGAAAGTGCTCATCGGAATCGTTCTCGGGCGGAAAGACTCAAGGATCTGCCGC
TATTGAGATCCAGTTGCTATAGCCACTCTGCACCCAGTTGATCTTCAGCATTTACTTT
CACCAGCGTTGGGTGCAAAAACAGGCAAGCAAAATGCCCAAAGAAGGAAATGAGTGC
ACACGAAAATGTTGGATGCTCATACTCGCTTTCAATATTGAAGCATTATCAGGGTT
ACTAGTACGTCTCAAGGATAAGTAAGTAAATTAAAGGTACGGGAGGTATTGGACAGGCC
ATAAAATATCTTATTTCATTACATCTGTGTTGGTTTGTGAATCGATAGTACTAAC
ATACGCTCTCCATCAAAACAAAACAAACTAGCAAAATAGGCTGCCCCAGTGCA
AGTCAGGTGCCAGAACATTCTCT

b) pcDNA-C120-Fluc

This construct was used for transfection of VP-El222 stable cell line. The Firefly luciferase ORF (*luc2* from Promega) was cloned into the pcDNA-C120-empty vector using EcoRI (5'end) and XbaI (3'end).

Bold= restriction sites

Underlined= C120 repeats

Yellow= minimal TATA-box promoter

Green= Firefly Luciferase open reading frame

Vector sequence:

GACGGATCGGAGATCTCGCTAGCCTCGAGTAGGTAGCTTACTGCGTTAGGTAGC
CTTTAGTCCATGCGTTAGGTAGCCTTACTGCGTTAGGTAGCCTTACTGCG

TTATAGGTAGCCTTAGTCATGAAGCTTAGACACTAGAGGGTATATAATGGAAGCTCGACTTC
CAGCTGGCAATCCGGTACTGTTGGTAAAGGATCCACTAGTCCAGTGTGGTGGAATTGCCACC
 ATGGAAGATGCCAAAACATTAAGAAGGGCCAGCGCCATTCTACCCACTCGAACGACGGGACCG
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 CGACGCACATATCGAGGTGGACATTACCTACGCCAGACTTCGAGATGAGCGTTGGCTGGCA
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 TTTGCCACCCGGCTCAACGAGTACGACTTCGTGCCCGAGAGCTTCGACCGGGACAAAACCATC
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 CGCTATCCTCAGCGTGGTGCCATTTCACCAACGGCTTCGGCATGTTACCCACGCTGGTACTTG
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 TCTCATCGACAAGTACGACCTAACGAACTTGCACGAGATGCCAGCGGGGGCGCCGCTCAGC
 AAGGAGGTAGGTGAGGCCGCGCCAAACGCTTCCACCTACCGCATCCGCCAGGGCTACGGCC
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 CAAGGTGGTGCCCTCTCGAGGCTAACGGTGGACTTGGACACCAGTAAGACACTGGTGTG
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 AACCATGACCGAGAAGGAGATCGTGGACTATGTGCCAGCCAGGTTACAACCGCCAAGAAGCTG
 CGCGGTGGTGTGTTCGGACGGTGCCTAAAGGACTGACCGGCAAGTTGGACGCCCGCA
AGATCCCGAGATTCTCATTAAGGCCAACAGAAGGGCGGCAAGATGCCGTGTAATCTAGAGGGCC
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 TCCCCCGTGCCTCCTGACCCCTGGAAAGGTGCCACTCCACTGTCCTTCTTAATAAAATGAGG
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 GCGCGGGGGTGTGGTACCGCGACGCGTACCGCTACACTTGCAGCGCCCTAGCGCCCGC
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 TTGCACGCAGGTTCTCCGGCGCTGGGTGGAGAGGCTATTGGCTATGACTGGCACAACAGA
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CAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTG
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CATGCCGACGGCGAGGATCTGCTGACCCATGGCGATGCCCTGCCGAATATCATGGT
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CAAAACAGGAAGGCAAAATGCCGAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACT
CATACTCTCCTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATAC

ATATTGAATGTATTAGAAAAATAACAAATAGGGGTTCCGCGCACATTCCCCGAAAAGTGC
CACCTGACGTC

4. pcDNA-C120-mCherry construct

The mCherry ORF was cloned into the pcDNA-C120-empty vector using EcoRI (5'end) and XbaI (3'end).

Bold= restriction sites

Underlined= C120 repeats

Yellow= minimal TATA promoter

Blue= mCherry ORF

Vector sequence:

GACGGATCGGGAGATCTCGCTAGCCTCGAGTAGGTAGCCTT~~AGTCCATGCGTTA~~**TAGGTAGC**
CTTAGTCCATGCGTTA~~AGGTAGCCTT~~AGTCCATGCGTTA~~AGGTAGCCTT~~AGTCCATGCG
TTATAGGTAGCCTT~~AGTCCATGAA~~GCTT**AGACACTAGAGGGTATATA**ATGGAAGCTCGACTTC
CAGCTTGGCAATCCGGTACTGTTGGTAAAGGATCCACTAGTCCAGTGTGGTG**GAATT**CGCCACC
ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTT~~CATGCGCTTCAAGGTGC~~
ACATGGAGGGCTCCGT~~GAACGGCCACGAGTT~~CGAGATCGAGGGCGAGGGCGAGGCCGCCCCTA
CGAGGGCACCCAGACGCCAAGCT~~GAAGGTGACCAAGGGTGGCCCCCTGCCCTCGCCTGGGAC~~
ATCCTGTCCCCTCAGTT~~CATGTACGGCTCCAAGGCCTACGT~~GAAGCACCCGCCGACATCCCCG
ACTACTTGAAGCTGT~~CCCTCCCCGAGGGCTTCAAGTGGGAGCGCGT~~GATGAACTTCGAGGACGG
CGGCGTGGTGACCGTGACCCAGGACTCCTCCCTG~~CAGGACGGCGAGTT~~CATCTACAAGGTGAAG
CTGCGGGCACCAACTTCCCCTCCGACGGCCCCGTAATG~~CAGAAGAACGACATGGGCTGGGAGG~~
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GCTGAAGGACGGCGGCCACTACGACGCTGAGGT~~CAAGACCACCTACAAGGCCAAGAACCGCGTG~~
CAGCTGCCGGCGCCTACAACGT~~CAACATCAAGTGGACATCACCTCCCACAACGAGGACTACA~~
CCATCGTGAACAGTACGAACCGCGAGGGCGCCACTCCACCGCGG~~CATGGACGAGCTGTA~~
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CAGCAACCAGGTGTGGAAAGTCCCCAGGCT~~CCCAGCAGGAGTATGCAAAGCATGCATCT~~
CAATTAGTCAGCAACCATA~~GTCCCCCTTAAC~~CCGCCCCATCCGCCCCCTAAC~~TCCGCCAGT~~
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CTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGG~~CTTTGGAGGCCTAGGCTTTGCAAAA~~

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CGTTTCCATAGGCTCCGCCCTGACGAGCATCACAAATGACGCTCAAGTCAGAGGTG
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AACCCGGTAAGACACGACTATGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGA
GGTATGTAGGCGGTGCTACAGAGTTCTGAAAGTGGCTAACACTACGGCTACACTAGAAC
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CCATCCGTAAGATGCTTTCTGACTGGTAGTACTCAACCAAGTCATTCTGAGAATAGGTA
TGC GGCGACCGAGTTGCTCTGCCGGCGTCAATACGGGATAATACCGGCCACATAGCAGAAC
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 CCAGCGTTCTGGGTGAGCAAAACAGGAAGGAAAATGCCGAAAAAAGGAATAAGGGCGAC
 ACGGAAATGTTGAATACTCATACTCTCCTTTCAATATTATTGAAGCATTATCAGGGTTAT
 TGTCTCATGAGCGGATACATATTGAATGTATTAGAAAATAACAAATAGGGTCCCGCGCA
 CATTCCCCGAAAAGTGCCACCTGACGTC

5. p(UAS)₅-Fluc construct

This construct was used for transient transfections. Five consensus GAL4 binding sites (amplified from pG5-SEAP vector (Clontech)) were cloned into the pGL4.23 vector (Promega) using KpnI (5'end) and HindIII (3'end).

Bold= restriction sites

Underlined= UAS repeats

Yellow= minimal TATA-box promoter

Green= Firefly Luciferase open reading frame

Vector sequence:

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GGCCTAACTGGCCGGTACCATGACCATGCGCCAAGCTAATTCCGGATCCGCCTCGG
AGGACAGTACTCCGCTCGGAGGACAGTACTCCGCTCGGAGGACAGTACTCCGCTCGAGGACAGT
ACTCCGCTCGGAGGACAGTACTCCGATCCGTCAGTCTAGCAAGCTTAGACACTAGAGGGTATA
TAATGGAAGCTCGACTTCCAGCTTGGCAATCCGGTACTGTTGGTAAAGCCACCATGGAAGATGC
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AAGGCCAGAAAAGGCCAGGAACCGTAAAAGGCCGTTGCTGGCTTTCCATAGGCTCCG
CCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCAAACCCGACAGGACTA
TAAAGATACCAGCGTTCCCCCTGGAAGCTCCCTCGGAAAGCGTGGCCTTCTCATAGCTCACGCTG
TTACCGGATACCTGTCCGCTTCTCCCTCGGAAGCTCCCTCGGAAAGCGTGGCCTTCTCATAGCTCACGCTG
TAGGTATCTCAGTCGGTAGGTCGCTCCAGCTCCAGTGGTAACTATCGTCTGAGTCCAACCCGTAAGACACGACT
TATGCCACTGGCAGCAGCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCAGGTGCTAC
AGAGTTCTGAAGTGGTGGCTAACTACGGCTACACTAGAACAGTATTGGTATCTCGC
CTGCTGAAGCCAGTTACCTCGGAAAAGAGTTGGTAGCTCTGATCCGGCAAACAAACCACCG
CTGGTAGCGGTGGTTTTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTAAGA
AGATCCTTGATCTTCTACGGGCTGACGCTCAGTGGAAACGAAACTCACGTTAAGGGATT
TTGGTATGAGATTATCAAAAGGATCTCACCTAGATCCTTAAATTAAAATGAAGTTTA
AATCAATCTAAAGTATATGAGTAAACTTGGTCTGACAGCGGCCGAAATGCTAAACCAC
AGTGGTTACAGTCTGATCAGTGAGGCACCGATCTCAGCGATCTGCCATTTCGTTGCT
TAGTGGCTGACTCCCCGTCGTAGATCAGTGGCTACCGATCTCAGCGATCTGCCATT
CGCAGCAATGATGCCGCGAGAGCCGTTACCGGCCCCGATTGTCAGCAATGAACCA
GCAGGGAGGGCCGAGCGAAGAAGTGGCTCTGCTACTTGTCCGCTCCATCCAGTCTATGAGCT
GCTGCTGATGCTAGAGTAAGAACAGTTGCCAGTGAGTAGTTCCGAAGAGTTGGCATT
TACTGGCATCGTGGTATCACGCTCGTCCGGTATGGCTCGTTCAACTCTGGTCCCAGCG
TCAAGCCGGTCACATGATCACCATATTATGAAGAACAGTCAGCTCCTAGGGCTCCGA
TCGTTGTCAGAAGTAAGTGGCCGGTGTGCTCATGGTAATGGCAGCACTACACA
TCTTACCGTCATGCCATCCGTAAGATGCTTCCGTGACCGGGAGTACTCAACCAAGT
TGTGAGTAGTGTATACGGCGACCAAGCTGCTCTGCCGGCTATACGGGACA
CACATAGCAGTACTTGAAAGTGCCTCATCGGAATCGTTCTCGGGCGAAAGACT
GATCTGCGCTATTGAGATCCAGTTCGATATAGCCACTCTGCACCCAGTTGAT
TCTTTACTTCACCAGCGTTGGGGTGTGCAAAACAGGCAAGCAAATGCC
GAATGAGTGCAGACACGAAATGTTGGATGCTCATACTCGCTTCAATT
TTATCAGGGTTACTAGTACGTCTCAAGGATAAGTAAGTAATATT
GACAGGCCGCAATAAAATCTTATTTCATTACATCTGTGTTGGTTTTGTG
ATAGTACTAACATACGCTCTCCATCAAAACAAACAAACTAGCAA
TCCCCAGTGCAAGTGCAGGTGCCAGAACATTCTCT

6. pVP-empty construct

Purchased from Clontech

Yellow= nuclear localization sequence

Green= VP16 activation domain

Vector sequence:

TATGTATCATACACATACTGATTAGGTGACACTATAGAACTCGACTGTGGAATGTGTGTCAGTT
AGGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAG
TCAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATC
TCAATTAGTCAGCAACCATACTCCGCCCTAATCCGCCATCCGCCCTAATCCGCCAG
TTCCGCCATTCTCCGCCCATGGCTGACTAATTTTTATTATGCAGAGGCCGAGGCC
TCGGCCTCTGAGCTATTCCAGAAGTAGTGAAGAGGCTTTGGAGGAGATCTAAGCTAGGCC
GCCACCATGGGCCCTAAAAAGAACGTAAGTCGCCCGACCGATGTCAGCCTGGGGACG
AGCTCCACTTAGACGGCGAGGACGTGGCGATGGCGATGCCAGCGCTAGACGATTCGATCT
GGACATGTTGGGGACGGGGATTCCCCGGGGCGGGATTACCCCCACGACTCCGCCCTAC
GGCGCTCTGGATATGGCCACTTCGAGTTGAGCAGATGTTACCGATGCCCTGGAATTGAGC
AGTACGGTGGGAATTCCCGGGATCCGTCACCGCTGCAGAAGCTTAGATAAGTAAGTA
ATGATCATAATCAGCCATACCACATTGTAGAGGTTACTTGCTTAAAAACCTCCCACACC
TCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTAACTTGTTATTGCAGCTTA
TAATGGTTACAAATAAAGCAATAGCATCACAAATTCAAAATAAAGCATTTCAGTGCAT
TCTAGTTGTGGTTGTCCAAACTCATCAATGTATCTTATCATGTCGGATCTGCCGGTCTCCCT
ATAGTGAGTCGTATTAATTGATAAGCCAGGTTAACCTGCATTAATGAATCGCCAACGCG
GGGAGAGGCAGGTTGCGTATTGGCGCTCTCCGCTTCCTCGCTCACTGACTCGCTGCCTCG
TCGTTCGGCTGCGCGAGCGGTATCAGCTACTCAAAGCGGTAAACGGTTATCCACAGAAC
AGGGGATAACGCAGGAAAGAACATGTGAGCAGGAAAGGCCAGGAAACCGTAAAAG
GCCGCGTTGCTGGCGTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCT
CAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAAGCGTTCCCGCTGGAAAGCTC
CCTCGTGCCTCTCGTCCGACCCCTGCCCTACGGGATACCTGTCGGCTTCTCCCTCG
GGAAGCGTGGCGCTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTAGGTCGTTGCT
CCAAGCTGGCTGTGTGCACGAACCCCCGTTAGCCGCTGCCCTATCCGTAAC
TCGTCTTGAGTCCAACCCGTAAGACACGACTTATGCCACTGGCAGCAGCCACTGGTAACAGG
ATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTGAAGTGGTGGCTAACTACGGCT
ACACTAGAAGGACAGTATTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGT
TGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTGTTGCAAGCAG
CAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTGATCTTCTACGGGTCTGACG
CTCAGTGGAACGAAACTCACGTTAAGGGATTTGGTCATGAGATTATCAAAAGGATCTCAC
CTAGATCCTTTAAATTAAAAATGAAGTTAAATCAATCTAAAGTATATGAGTAAACCTGG
TCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGCTATTCGTTCAT
CCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATAACGGGAGGGCTTACCATCTGCC
CAGTGCCTGCAATGATACCGCGAGACCCACGCTCACCGCTCCAGATTATCAGCAATAACCAG
CCAGCCGGAAGGGCGAGCGCAGAAGTGGCTCTGCAACTTATCCGCTCCATCCAGTCTATT
ATTGTTGCCGGGAAGCTAGAGTAAGTAGTCGCCAGTTAATAGTTGCGCAACGTTGTTGCCAT
TGCTACAGGCATCGTGGTGACGCTCGTCGTTGGTATGGCTTCATTAGCTCCGGTCCCAA
CGATCAAGGCAGTTACATGATCCCCATGTTGCAAAAAAGCGGTTAGCTCCTCGGTCTC
CGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTATCACTCATGGTTATGGCAGCAGTGCATAA
TTCTCTTACTGTCATGCCATCCGTAAGATGCTTTCTGTGACTGGTGAGTACTCAACCAAGTCA
TTCTGAGAATAGTGTATGCGGCAGCGAGTTGCTCTGCCCCGGCTCAATACGGGATAATACCG
CGCCACATAGCAGAACTTAAAGTGCATCATTGGAAAACGTTCTCGGGCGAAAACCTCTC
AAGGATCTTACCGCTGTTGAGATCCAGTTGATGTAACCCACTCGTGCACCCAACTGATCTCA
GCATCTTTACTTCACCAGCGTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGAAAAA

AGGGAAATAAGGGCGACACGAAATGTTGAATACTCATACTCTTCCTTTCAATATTATTGAAG
CATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTGAATGTATTAGAAAAATAACAA
ATAGGGGTTCCCGGCACATTCCCCAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCA
TGACATTAACCTATAAAATAGGCGTATCAGGAGGCCCTTCGTCTCGCGCGTTCGGTGATGA
CGGTGAAAACCTCTGACACATGCAGCTCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCC
GGGAGCAGACAAGCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGGCTGGCTTAAC
ATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGGACATATTGTCGTTAGAACCGG
CTACAATTAAATACATAACCT

7. pVP-EL222 construct

The EL222 ORF (amino acids 14-222) was cloned into the pVP16 vector (Clontech) using EcoRI (5'end) and SpeI/XbaI (3'end).

Bold= restriction sites

Yellow= nuclear localization sequence

Green= VP16 activation domain

Blue= EL222 sequence (aa 14-222)

Vector sequence:

TATGTATCATACACATACGATTTAGGTGACACTATAGAACTCGACTGTGGAATGTGTGTCAGTT
AGGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGAGAAGTATGCAAAGCATGCATCTCAATTAG
TCAGCAACCAGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGAGAAGTATGCAAAGCATGCATC
TCAATTAGTCAGCAACCATACTCCGCCCTAATCCGCCATCCGCCCTAATCCGCCAG
TTCCGCCCTATTCTCCGCCCTATGGCTGACTAATTTTTTATTATGCAGAGGCCGAGGCC
TCGGCCTCTGAGCTATTCCAGAAGTAGTGAAGAGGCTTTGGAGGAGATCTAAGCTAGGCC
GCCACCATGGGCCCTAAAAAGAAGCGTAAAGTCGCCCGACCGATGTCAGCCTGGGGACG
AGCTCCACTTAGACGGCGAGGACGTGGCGATGGCGATGCCGACGCGCTAGACGATTCGATCT
GGACATGTTGGGGACGGGGATTCCCCGGGGCGGGATTACCCCCCACGACTCCGCCCTAC
GGCGCTCTGGATATGGCCGACTTCGAGTTGAGCAGATGTTACCGATGCCCTTGAATTGACG
AGTACGGTGGG GAATTC GGGCAGACGACACACGCGTTGAGGTGCAACCGCCGGCGAGTGGT
CCTCGACCTGATCGAGGCCAGCCCAGCGATCGCATCGCTGTCCGATCCGCTCGCCACAAT
CCGCTGATGCCATCAACCAGGCCTCACCGACCTGACCGGCTATTCCGAAGAAGAATGCGTCG
GCCGCAATTGCCGATTCTGGCAGGTTCCGGCACCGAGCCGTGGCTGACCGACAAGATCCCCA
AGCGTGCAGGACAAGCCGGTCTGGTCAGATCCTGAACACTACAAGAAGGACGGCACGCC
TTCCGCAATGCCGTGCTCGTGCACCGATCTACGATGACGACGAGCTCTATTCCCG
GCAGCCAGGTGCAAGTCGACGACGACCGACAGCCAACATGGCATGGCGCCGCAACGCC
GGAAATGCTCAGGACGCTGCGCCGCCAGCTCGAGGTTACGACGCTGGCATGGCTTG
CGCAACAAGGAAGTGGCGGCCGGCTCGGCCTGTCGGAGAAAACCGTCAAGATGCACCGCG
TGGTGATGGAAAAGCTAACCTGAAGACCAAGTGCACGATCTGGTGCCTGGCGATTGCC
AATCTAA**ACTAGATAAGTAATGATCATAATCAGCCATACCACATTGTAGAGGTTTACTGCT**
TTAAAAAAACCTCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTA
ACTTGTTATTGCAGCTATAATGGTTACAAATAAGCAATAGCATCACAAATTCAAAATAA
AGCATTTCCTACTGCATTCTAGTGTGGTTGTCCAAACTCATCAATGTATCTTATCATGTC
TGGATCTGCCGTCTCCCTATAGTGAGTCGTTAATTGATAAGCCAGGTTAACCTGCATTA
ATGAATCGGCCAACGCGCGGGAGAGGCAGGTTGCGTATTGGCGCTTCCGCTTCGCTC

ACTGACTCGCTCGCTCGGCTGGCTGCGAGCGGTATCAGCTCACTCAAAGGCAGTAA
 TACGGTTATCCACAGAATCAGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAA
 GGCCAGGAACCGTAAAAGGCCGCTGCTGGCTTTCCATAGGCTCCGCCCCCTGACGAG
 CATCACAAAATCGACGCTCAAGTCAGAGGTGGGAAACCCGACAGGACTATAAAGATACCAGG
 CGTTCCCCCTGGAAGCTCCCTCGTGCCTCCTGTTCCGACCCCTGCCGTTACCGGATACCT
 GTCCGCCTTCTCCCTCGGAAGCGTGGCGCTTCTCAATGCTCACGCTGTAGGTATCTCAGT
 TCGGTGTAGGTCGTCGCTCCAAGCTGGCTGTGCACGAACCCCCGTTAGCCGACCGCT
 GCGCCTTATCCGGAACACTATCGTCTGAGTCAACCCGTAAGACACGACTTATGCCACTGGC
 AGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTGAAG
 TGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTGGTATCTGCGCTTGCTGAAGCCAG
 TTACCTTCGGAAAAAGAGTTGGTAGCTCTGATCCGAAACAAACCACCGCTGGTAGCGGTGG
 TTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTGATC
 TTTTCTACGGGCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTGGTATGAGAT
 TATCAAAAAGGATCTTCACCTAGATCCTTTAAATTAAAATGAAGTTTAAATCAATCTAAAG
 TATATATGAGTAAACTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTAGCG
 ATCTGTCTATTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTAGATAACTACGATACGGG
 AGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGA
 TTTATCAGCAATAAACCCAGCCAGCCGAAGGGCGAGCGCAGAAGTGGCCTGCAACTTATCC
 GCCTCCATCCAGTCTATTAAATTGTTGCCATTGCTACAGGCATCGTGGTGTGACGCTCGTGTGTTGGTATGGCTTC
 ATTCAAGCTCCGGTCCCACGATCAAGGCAGTTACATGATCCCCATGTTGTGCAAAAAAGCG
 GTTAGCTCCTCGGTCCCTCGATCGTGTAGAAGTAAGTTGGCCGAGTGTATCACTCATGG
 TTATGGCAGCACTGCATAATTCTCTACTGTCATGCCATCCGTAAGATGCTTTCTGTGACTGG
 TGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGGCGGACCGAGTTGCTCTGCCGGCG
 TCAATAACGGATAATACCGGCCACATAGCAGAACTTTAAAAGTGTCTCATCATTGAAAACGTT
 CTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTGATGTAACCCACTCG
 TGCACCCAACGTCTCAGCATCTTACTTACCCAGCGTTCTGGGTGAGCAAAACAGGA
 AGGCAAAATGCCGAAAAAGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTCC
 TTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCATGAGCGGATACATATTGAATG
 TATTAGAAAAATAACAAATAGGGTTCCCGCACATTCCCCGAAAAGTGCCACCTGACGTC
 TAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCAGGAGGCCCTTCGTC
 TCGCGCTTCCGGTATGACGGTAAAACCTCTGACACATGCAGCTCCGGAGACGGTCACAGC
 TTGTCTGTAAGCGGATGCCGGAGCAGACAAGCCGTCAGGGCGCGTCAGCGGGTGTGGCGGG
 TGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGACTGAGAGTGCACCATATGGACA
 TATTGTCGTTAGAACGCGGCTACAATTAAATACATAACCT

8. pIRES-puro-VP-EL222 construct

The VP-EL222 ORF from the pVP-EL222 vector was cloned into the pIRES-puro vector (Clontech) using EcoRV (5'end) and BamHI (3'end).

Bold= restriction sites

Yellow= nuclear localization sequence

Green= VP16 activation domain

Blue= EL222 sequence (aa 14-222)

Vector sequence:

GACGGATCGGGAGATCTCCGATCCCCATGGTCGACTCTCAGTACAATCTGCTCTGATGCCGC
 ATAGTTAACGCAGTATCTGCTCCCTGCTGTGGAGGTCGCTGAGTAGTGCGCAGCAAA
 ATTTAAGCTACAACAAGGCAAGGCTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGC
 GTTTGCGCTGCTCGCATGTCAGGGCCAGATAACCGTTGACATTGATTATTGACTAGTTA
 TTAATAGTAATCAATTACGGGGTCATTAGTCATAGCCCATAATGGAGTTCCGCGTTACATAA
 CTTACGGTAAATGCCCGCTGGCTGACCGCCAACGACCCCCGCCATTGACGTCAATAATGA
 CGTATGTTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTGGACTATTACG
 GTAAACTGCCCACTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTC
 AATGACGGTAAATGCCCGCTGGCATTATGCCAGTACATGACCTATGGGACTTTCTACTT
 GGCAGTACATCTACGTATTAGTCATCGTATTACCATGGTATGCCGTTGGCAGTACATCAA
 TGGCGTGGATAGCGGTTGACTCACGGGATTTCACAAGTCTCCACCCATTGACGTCAATGGG
 AGTTTGTGTTGGCACCAAAATCAACGGGACTTCCAAAATGTCGTAACAACACTCCGCCCCATTGA
 CGCAAATGGCGGTAGGCAGTACGGTGGGAGGTCTATATAAGCAGAGCTCTGGCTAACTAG
 AGAACCCACTGCTTACTGGCTTATGAAATTAAATACGACTCACTATAGGGAGACCAAGCTTGG
 TACCGAGCTCGGATC**GATATC**ATGGGCCTAAAAAGAAGCGTAAAGTCGCCCGACCGATG
 TCAGCCTGGGGACGACTCCACTTAGACGGCAGGACGTGGCATGGCGATGCCGACCGCCT
 AGACGATTCGATCTGGACATGTTGGGGACGGGGATTCCCCGGGGGGGATTACCCCCAC
 GACTCCGCCCCCTACGGCGCTCTGGATATGCCGACTTCGAGTTGAGCAGATGTTACCGATG
 CCCTTGGAAATTGACGAGTACGGTGGGAATTCCGGGAGACGACACACCGTTGAGGTGCAACC
 GCCGGCGAGTGGTCCTCGACCTGATCGAGGCCAGCCGATCGCATCGTCGTCCGATCCG
 CGTCTCGCGACAATCCGCTGATGCCATCAACCAGGCCTCACCGACCTGACCGGCTATTCCG
 AAAAGAAGATGCGTCGGCGCAATTGGCATTCCGGAGGTTCCGGACCGAGCCGTGGCTGAC
 CGACAAGATCCGCAAGGCGTGCAGCACAAGCCGGTCTGGCGAGATCCTGAACACTACAAG
 AAGGACGGCACGCCGTTCCGCAATGCCGTGCTCGTGCACCGATCTACGATGACGAGCAGGAGC
 TTCTCTATTCCTCGGCAGCCAGGTGCAAGTCGACGACGACAGCCAACATGGCATGGCG
 CCGCGAACGCCGCCGGAAATGCTCAGGACGCTGTCGCCGCCAGCTCGAGGTTACGACGCTG
 GTGGCATGGGCTTGCACAAAGGAAGTGGCGCCGGCTGGCTGTGGAGAAAACCGTCA
 AGATGCACCGCGGGCTGGTATGGAAAAGCTAACCTGAAGACCAAGTGGCGATCTGGTGCGCAT
 TGCCGTGAAAGCCGAATCTAA**ACTAGA****GGATCC**ACTAGTAACGGCCAGTGTGCTGGAATT
 ATTTCGCTGTCTCGAGGGCCAGCTGTTGGGGTGAGTAGTACTCCCTCTAAAAGCGGGCATGACTT
 CTGCGCTAAGATTGTCAGTTCCAAAACGAGGAGGATTGATATTCACCTGGCCCGGGTGT
 GCCTTGAGGGTGGCGCGTCCATCTGGTCAAGAAAAGACAATCTTTGTTGTCAAGCTGAGG
 TGTGGCAGGCTTGAGATCTGCCATACACTTGAGTGACATGACATCCACTTGCTTCTCTC
 CACAGGTGTCACCTCCAGGTCCAAGTGCAGGTGAGCATGCATCTAGGGCGGCCATTCCGCC
 CCTCTCCCTCCCCCCCCCTAACGTACTGGCGAAGCCGCTGGATAAGGCCGTGCGTT
 TGTCTATATGTGATTTCCACCATATTGCCGTCTTTGGCAATGTGAGGGCCGGAAACCTGGC
 CCTGCTTCTTGACGAGCATTCTAGGGTCTTCCCTCTGCCAAAGGAATGCAAGGTCTGT
 TGAATGTCGTGAAGGAAGCAGTCCCTGTGAAGCTTCTGAAGACAAACAACGTCTGTAGCGAC
 CCTTGCAGGAGCGGAACCCCCACCTGGCGACAGGTGCTCTGCCAAAAGCCACGTGTA
 TAAGATAACCTGCAAGGGCAGCAACCCCCAGTGCCACGTTGAGTTGGATAGTTGTGGAAA
 GAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGCTGAAGGATGCCAGAAGGTACCCCA
 TTGTATGGATCTGATCTGGGCCTCGGTGACATGCTTACATGTGTTAGTCGAGGTTAAAAA
 AAACGTCTAGGCCCCCGAACACGGGACGTGGTTCTTCAAGGAAACACGATGATAAGCTT
 GCCACAACCCGGATGACCGAGTACAAGCCCACGGTGCACGCCACCCGCGACGACGTCCCC
 AGGGCGTACGCACCCCTGCCGCCGTTGCCGACTACCCGCCACGCCACACCGTGCATC
 CGGACGCCACATCGAGCGGGTACCGAGCTGCAAGAACTCTTCCTCACGCCGTGGCTCGA
 CATCGGCAAGGTGTGGTGCACGCCGACGGCGCCGCGGTCTGGACCACGCCGGAGAGC

GTCGAAGCGGGGGCGGTGTTGCCGAGATCGGCCGCATGCCGAGTTGAGCGGTTCCCGGC
TGGCCGCGCAGCAACAGATGGAAGGCCCTGGCGCCGCACCGGCCAAGGAGCCGCGTGGTT
CCTGGCCACCGTGGCGTCTGCCCGACCACCAAGGGCAAGGGTCTGGGAGCGCCGTCGTGCTC
CCCGGAGTGGAGGGCGGAGCGGCCGGGTGCCGCCCTGGAGACCTCCCGCCCCGCA
ACCTCCCCTCTACGAGCGCTCGGTTCACCGTCACCGCCACGTCGAGGTGCCGAAGGACC
GCGCACCTGGTGCATGACCCGCAAGGCCGGTGCCTGACTCTAGAGCTCGCTGATCAGCCTCGAC
TGTGCCTCTAGTTGCCAGCCATCTGTTGCCCTCCCCGTGCCTCCTGACCCGGAA
GGTGCCACTCCCAGTCCCTTAATAAAATGAGGAAATTGCATCGCATTGAGTAGGT
GTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAG
CAGGCATGCTGGGATGCGGTGGCTCTATGGCTCTGAGGCGGAAAGAACAGCTGGGCTCG
AGTGCATTCTAGTTGTGGTTGTCAAACACTCATCAATGTATCTTATCATGCTGTATACCGTCG
ACCTCTAGCTAGAGCTTGGCGTAATCATGGTCATAGCTGTTCCGTGAAATTGTTATCCGC
TCACAATTCCACACAACATACGAGCCGAAGCATAAAGTGTAAAGCCTGGGTGCCTAATGAGT
GAGCTAACTCACATTAATTGCGTTGCGCTACTGCCGCTTCCAGTCGGGAAACCTGTCGTG
CAGCTGCATTAATGAATCGCCAACGCGCGGGAGAGGCGGTTGCGTATTGGCGCTTCCG
CTTCCTCGCTCACTGACTCGCTCGTCGGTCGGCTGCGAGCGGTATCAGCTCACTC
AAAGGCAGTAATACGGTTATCCACAGAATCAGGGATAACGCAAGGAAAGAACATGTGAGCAAAA
GGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCTTTCCATAGGCTCCGCC
CCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATA
AAGATACCAGGCAGTCCCTGGAAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCGTGCCGCTT
ACCGGATACTGTCGCCCTTCTCCCTCGGAAGCGTGGCGCTTCTCAATGCTCACGCTGTA
GGTATCTCAGTCGGTGTAGGTCGCTCCAAGCTGGCTGTGCAACGAACCCCCGTTCA
GCCCGACCGCTGCCCTATCCGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTA
TCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAG
AGTTCTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTGGTATCTGCGCTCT
GCTGAAGCCAGTTACCTTCCGAAAAAGAGTTGGTAGCTCTGATCCGCAAACAAACCACCGCT
GGTAGCGGTGGTTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAG
ATCCTTGATCTTCTACGGGCTGACGCTCAGTGGACAGAAAACACGTTAACGGATT
GGTCATGAGATTATCAAAAAGGATCTCACCTAGATCCTTAAATTAAAAATGAAGTTAAA
TCAATCTAAAGTATATGAGTAAACTTGGCTGACAGTTACCAATGCTTAATCAGTGAGGCAC
CTATCTCAGCGATCTGTCTATTCGTTCATCCATAGTGCCTGACTCCCCGTGTCAGATAAC
TACGATACTGGAGGGCTTACCATCTGGCCCCAGTGCATGACGAACTACGCTCA
CCGGCTCCAGATTATCAGCAATAAACAGCCAGCCAGCGGAAGGGCGAGCGCAGAAGTGGTCTG
CAACTTATCCGCTCCATCCAGTCAGTCTATTAAATTGTTGCCGGGAAGCTAGAGTAAGTAGTCGCC
AGTTAATAGTTGCGCAACGTTGCTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTT
GGTATGGCTTCATTCAAGCTCCGGTCCCAACGATCAAGGCAGTTACATGATCCCCATGTT
GCAAAAAAGCGGTTAGCTCCTCGGTCCGATCGTGTAGAAGTAAGTGGCCGCAGTGT
ATCACTCATGGTTATGGCAGCACTGCATAATTCTTACTGTCATGCCATCCGTAAGATGCTTT
TCTGTGACTGGTGGACTCAACCAAGTCATTGAGAATAGTGTATGCGGCGACCGAGTTGCT
CTTGGCCGGCGTCAACAGGGATAATACCGGCCACATAGCAGAACTTAAAAGTGCATCAT
TGGAAAACGTTCTCGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATG
TAACCCACTCGTGCACCCAACTGATCTCAGCATCTTACTTCAACAGCGTTCTGGGTGAG
CAAAACAGGAAGGCAAAATGCCGAAAAAAGGAATAAGGGCGACACGGAAATGTTGAATACT
CATACTCTCCTTTCAATATTATGAAAGCATTATCAGGGTTATTGTCATGAGCGGATAC
ATATTGAAATGTATTAGAAAAATAACAAATAGGGTTCCGCGCACATTCCCCGAAAAGTGC
CACCTGACGTC

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